PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
74618-16		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/00255	10/03/2000	11/03/1999
Applicant		
THE UNIVERSITY OF MANITOBA et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

[ ] the text is approved as submitted by the applicant.

[X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 00/00255

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9, 14-19, 36, 37, 39, 40 (all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00255

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/04 A61K31/195 A61K31/295 A61K31/70 A61K31/415
A61K31/535 A61K31/145 A61K31/40 A61K31/10 A61K38/44
A61K35/34 C12N5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ULIBARRI J A ET AL: "Nitric oxide stimulates myoblast proliferation in vitro." MEDICINE AND SCIENCE IN SPORTS AND EXERCISE, vol. 29, no. 5 SUPPL., 1997, page S228 XP000961780 44th Annual Meeting of the American College of Sports Medicine;Denver, Colorado, USA; May 28-31, 1997 ISSN: 0195-9131 abstract --- -/--	1-12,36, 40

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 December 2000	

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	A. Jakobs

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00255

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US1993 BAEK MI-YEONG ET AL: "Changes in the cellular cGMp levels and guanylate cyclase activities during chick myoblast fusion." Database accession no. PREV199396097003 XP002154299 abstract & KOREAN JOURNAL OF ZOOLOGY, vol. 36, no. 3, 1993, pages 433-438, ISSN: 0440-2510	1-9, 14-19, 36,37,40
X	BREDT DAVID S: "NO skeletal muscle derived relaxing factor in Duchenne muscular dystrophy." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 25, December 1998 (1998-12), pages 14592-14593, XP000960480 Dec., 1998 ISSN: 0027-8424 page 14592, column 2, paragraph 3 -page 14593, column 1, paragraph 5	1
X	SARKAR RAJABRATA ET AL: "Nitric oxide inhibition of endothelial cell mitogenesis and proliferation." SURGERY (ST LOUIS), vol. 118, no. 2, 1995, pages 274-279, XP000961764 ISSN: 0039-6060 abstract	1
X	DATABASE WPI Section Ch, Week 199831 Derwent Publications Ltd., London, GB; Class B03, AN 1998-350696 XP002154301 & JP 10 120654 A (ONO PHARM CO LTD), 12 May 1998 (1998-05-12) abstract	1

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00255

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

	Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
1	X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US November 1997 (1997-11) LAMOSOVA D ET AL: "Influence of melatonin on chick skeletal muscle cell growth." Database accession no. PREV199800098087 XP002154300 abstract & COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY C PHARMACOLOGY TOXICOLOGY &, vol. 118, no. 3, November 1997 (1997-11), pages 375-379, Nov., 1997 ISSN: 0742-8413	1
4	X	--- AZZENA G B ET AL: "NITRIC OXIDE REGENERATES THE NORMAL COLONIC PERISTALTIC ACTIVITY IN MDX DYSTROPHIC MOUSE" NEUROSCIENCE LETTERS,LIMERICK,IE, vol. 261, no. 1/02, 1999, pages 9-12, XP000879028 ISSN: 0304-3940 the whole document	1-9, 14-19, 36,37, 39,40
1	X	--- LEE KUN HO ET AL: "Nitric oxide as a messenger molecular for myoblast fusion." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 20, 1994, pages 14371-14374, XP002154298 ISSN: 0021-9258 abstract; figures 1,4 page 14372, column 2, paragraph 3	1-9, 14-19, 36,37,40
1	X	--- YAN ZHONG-QUN ET AL: "Overexpression of inducible nitric oxide synthase by neointimal smooth muscle cells." CIRCULATION RESEARCH, vol. 82, no. 1, pages 21-29, XP000961767 ISSN: 0009-7330 abstract page 24, column 2, paragraph 5 -page 26, column 2, paragraph 1; figures 7,8 page 28, column 2, paragraphs 2,3	1-9, 14-19, 36,37,40
		--- -/--	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00255

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

	Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
4	X	HAYCOCK J W ET AL: "OXIDATIVE DAMAGE TO MUSCLE PROTEIN IN DUCHENNE MUSCULAR DYSTROPHY" NEUROREPORT,GB,RAPID COMMUNICATIONS OF OXFORD, OXFORD, vol. 8, no. 1, 1996, pages 357-361, XP000879014 ISSN: 0959-4965 abstract page 357, column 2, paragraph 2 -page 358, column 1, paragraph 1 page 361, column 1, paragraph 2 -column 2, paragraph 2 ---	1-9, 14-19, 36,37, 39,40
1	X	CHAO DANIEL S ET AL: "Selective loss of sarcolemmal nitric oxide synthase in becker muscular dystrophy." JOURNAL OF EXPERIMENTAL MEDICINE, vol. 184, no. 2, 1996, pages 609-618, XP000961763 ISSN: 0022-1007 abstract page 610, column 1, paragraphs 2,3 table 1 page 616, column 2, paragraphs 2,3 ---	1-9, 14-19, 36,37, 39,40
1	X	AZZENA GIAN BATTISTA ET AL: "Nitric oxide regenerates the normal colonic peristaltic activity in mdx dystrophic mouse." NEUROSCIENCE LETTERS, vol. 261, no. 1-2, 12 February 1999 (1999-02-12), pages 9-12, XP000961771 ISSN: 0304-3940 abstract page 9, column 1 -page 10, column 1, paragraph 1 page 12, column 1 ---	1-9, 14-19, 36,37, 39,40
3	X	US 5 583 101 A (STAMLER JONATHAN ET AL) 10 December 1996 (1996-12-10) abstract; examples 1-6 column 1 -column 6, line 40 column 8, paragraph 2 ---	1-9, 14-19, 36,37, 39,40

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00255

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SOHN YOON K ET AL: "Neuritic sprouting with aberrant expression of the nitric oxide synthase III gene in neurodegenerative diseases." JOURNAL OF THE NEUROLOGICAL SCIENCES, vol. 162, no. 2, 15 January 1999 (1999-01-15), pages 133-151, XP000961766 ISSN: 0022-510X the whole document	1-9, 14-19, 36,37, 39,40
A	WO 97 33173 A (UNIV CALIFORNIA) 12 September 1997 (1997-09-12) the whole document	1-40
P,X	KALIMAN, PERLA ET AL: "Insulin-like growth factor-II, phosphatidylinositol 3-kinase, nuclear factor-.kappa.B and inducible nitric-oxide synthase define a common myogenic signaling pathway" J. BIOL. CHEM. (1999), 274(25), 17437-17444, XP000960874 the whole document	1-9, 14-19, 36,37,40
A	EL-DADA, MANAR D. ET AL: "Involvement of nitric oxide in nicotinic receptor-mediated myopathy" J. PHARMACOL. EXP. THER. (1997), 281(3), 1463-1470, XP000972194 the whole document	1-40

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 00/00255

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 10120654 A	12-05-1998	NONE	
US 5583101 A	10-12-1996	AU 3008395 A	16-02-1996
		CA 2194991 A	01-02-1996
		JP 10511075 T	27-10-1998
		WO 9602241 A	01-02-1996
		US 5545614 A	13-08-1996
WO 9733173 A	12-09-1997	AU 2208097 A	22-09-1997

09/936609

PATENT COOPERATION TREATY

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PCT 14

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 03 JUL 2001
WIPO PCT

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
74618-16		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00255	10/03/2000	11/03/1999
International Patent Classification (IPC) or national classification and IPC A61K33/00		
Applicant THE UNIVERSITY OF MANITOBA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 32 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/10/2000	29.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Giacobbe, S Telephone No. +49 89 2399 8463

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00255

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

2,4-31,33-104	as originally filed			
105,106	with telefax of	06/10/2000		
1a,3,3a,32,32a	as received on	22/02/2001	with letter of	20/02/2001
1	with telefax of	15/06/2001		

Claims, No.:

1-76	with telefax of	15/06/2001

Drawings, sheets:

1/24-23/24	as originally filed	
24/24	with telefax of	06/10/2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 10, 20-24, 30-59, 64, 65, 68-74.

because:

☒ the said international application, or the said claims Nos. 1-22, 30, 31, 34, 35, 38, 39, 42, 43, 46, 47, 50, 51, 54-70, 73, 74 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 10, 20-24, 30-59, 64, 65, 68-74.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:
   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-9, 11-19, 60-63, 66 and 67.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-9, 11-19, 24-29, 60-63, 66 and 67
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-9, 11-19, 24-29, 60-63, 66 and 67
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	1-9, 11-19, 60-63, 66 and 67

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Section I

1.1 The amendments introduced on pages 105 and 106 and in Figure 14 do not fulfill the requirements set out in the PCT Guidelines (cf. VI-7.14) and cannot therefore be considered as correcting errors. Since moreover they completely reverse the conclusions to be drawn from the experiments, they are considered as introducing new subject-matter (see PCT Guidelines, VI-7.9) and are therefore not accepted.

1.2 The amended claims filed on 15.06.2001 do not fulfill the requirements of Art 34(2)(b) PCT since the concept of sustained skeletal muscle formation and/or repair was not present in the application as filed. The present Report has therefore been established based on the claims filed on 20.02.2001.

2. Section III

2.1 The present Opinion is based on a Partial Search Report where only claims 1-9, 14-19, 36, 37, 39 and 40 have been searched. These claims correspond to claims 1-9, 11-19, 60-63, 66 and 67 of 20.02.2001, and only these are therefore object of this Opinion (see Rules 66.1 (e) and 70.1 (d) PCT).

2.2 Claims 1-22, 30, 31, 34, 35, 38, 39, 42, 43, 46, 47, 50, 51, 54-70, 73 and 74 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT). However, although not required under the provisions of the PCT, an opinion will be given with respect to novelty and inventive step.

1. Section IV

This IPEA agrees with the objection as to lack of unity put forward by the ISA, for the reasons already given in Form PCT/ISA/206. Since the Applicant, upon invitation, has not paid any additional fee, the present Opinion will be drawn only with respect of the invention first mentioned in the application, i.e. the invention for which a Search Report has been established. This invention, concerned with the use of NO in the *in vivo* modulation of the activation of muscle precursor cells in relation to the treatment of dystrphies, is contained in claims 1-9, 11-19, 60-63, 66 and 67.

3. Section V

3.1 Cited Documents

The following documents (D) are referred to in this Report:

D1: WO 97 33173 A (UNIV CALIFORNIA) 12 September 1997

D2: US-A-5 583 101 (STAMLER JONATHAN ET AL) 10 December 1996

D3: DATABASE WPI Section Ch, Week 199831 Derwent Publications Ltd., London, GB; Class B03, AN 1998-350696, XP002154301

D4: LEE KUN HO ET AL, JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 20, 1994, pages 14371-14374, XP002154298

D5: DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US1993 BAEK MI-YEONG ET AL, XP002154299

D6: BREDT DAVID S, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 25, 1998, pages 14592-14593, XP000960480

3.2 Art 33(2) PCT (Novelty)

The subject-matter of present claims 1-9, 11-19, 24-29, 60-63, 66 and 67 does not meet the requirements of Art 33(2) PCT.

3.2a Document D1 discloses that NO and neuronal NO synthase can be used for the treatment of muscular dystrophies (cf. abstract; p. 2, ll. 1-12; and p. 11, ll. 15-26). This document is therefore novelty-destroying for claims 1-9, 11-19, 60-63, 66 and 67

n.b. The fact that NO and NO synthase are disclosed in D1 to act by a mechanism which has nothing to do with the activation of satellite cells is irrelevant, since not the mechanism of action but rather the treated diseases define the invention. In other words, the discovery of a new mechanism of action of a known substance used in the state of the art to treat a given disease is not a patentable invention unless it solves a specific technical problem (e.g. a specific time course of the treatment) over the same prior art. In such a case however the technical feature allowing the solution of this technical problem must be present in the application as filed and indicated in the claims: the mechanism of action does not constitute such a technical feature.

3.2b Document D2 discloses that pharmaceutical compositions containing NO synthase

inhibitors (cf. cols. 2-6) can be used for the treatment of muscular dystrophies (cf. col. 8, l. 15). This document is therefore (cf. n.b. above) novelty-destroying for claims 1-9, 11-19, 24-29, 60-63, 66 and 67.

3.2c Document D3 discloses that pharmaceutical compositions containing NO synthase inhibitors can be used for the treatment of muscular dystrophies. This document is therefore (cf. n.b. above) novelty-destroying for claims 1-9, 11-19, 24-29, 60-63, 66 and 67.

3.3 Art 33(3) PCT (Inventive step)

The subject-matter of present claims 1-9, 11-19, 24-29, 60-63, 66 and 67 does not meet the requirements of Art 33(2) PCT.

Document D4 (cf. p. 14371, col. 2, first complete paragraph) explicitly states that "the fusion of mononucleated myoblasts (i.e. of myogenic precursor cells or satellite cells as these cells are called in the present description, p. 1) into multinucleated myotubules (i.e. of myofibers, cf again the present description, p. 1)" constitutes a prominent event in the differentiation of embryonic muscle cells, an event which is shown in D4 itself, as well as in D5, to be mediated by NO. Furthermore it was known at the date of first filing of the present application that "muscle repair and formation are enabled by satellite (i.e. myoblast) activation" (cf. present description, p. 5, ll. 5-7). The skilled person could have therefore come to the logical conclusion that NO, by mediating myoblast fusion, could solve the technical problem of how to promote muscle repair and formation. This is confirmed by the conclusion contained in D6 that "manipulating NO levels in muscle may represent a possible strategy for the treatment of muscular dystrophy" (cf. p. 14593, last sentence).

3.4 Art 33(4) PCT (Industrial applicability)

As stated above, no opinion is given on the question of whether present claims 1-9, 11-19, 60-63, 66 and 67 are industrially applicable since their patentability is inter alia dependent upon their formulation as well as upon national and regional laws and no unifying criteria is provided in this field by the PCT.

4. Section VIII

Independent claims 1-3, 11-13 and 60-63 are not clear because they define the subject-matter to be protected by way of the biological mechanism underlying the

action of the disclosed compounds. If a claim is directed to a condition susceptible of being improved or prevented by selective interaction with a biological pathway, the claim can be regarded as clear only if instruction, in the form of experimental tests or any testable criteria, allowing the skilled person to recognise which conditions fall within the functional definition (and accordingly within the scope of the claims concerned) are available from the patent documents or from the general common knowledge. The selective interaction with a biological pathway itself cannot be considered as a therapeutic application. In the absence of such tests or criteria, a clear indication of the diseases to be treated is required in order to fulfill the requirements of Art 6 PCT. The claims have been examined under the assumption that the diseases indicated in claims 66 and 67 are intended.

In this experiment, muscle tissues are collected from the treated animals. LTA and diaphragm muscles were sectioned, and counts of fibers with central nuclei and fibers with peripheral nuclei were performed. The largest diameter of the muscle section in each of the two sections of each muscle was used for counting, for 10 mice per group (2 muscles per mouse).

Figure 14 shows that in the mdx mouse, the CNI in placebo-treated animals is about 0.6 (i.e. 60% of fibers) show a centrally located nucleus in a cross section of the muscle. This is similar for the tibialis anterior muscle (LTA) and diaphragm at the age shown in the graph (which is 8 weeks of age) and is reliably used to monitor the progressive effect of dystrophic fiber injury on a muscle over time as the disease progresses. CNI will increase with age in the mdx mice (until the plateau discussed above). Mice are treated from 4-8 weeks of age with placebo, Deflazacort, D+L-NAME or D+L-Arginine.

With deflazacort treatment for 4 weeks, the CNI is significantly less than in placebo-treated mdx LTA (down to 0.4 or 40%) in the left TA (LTA). The CNI in diaphragm (DIA) also decreases with deflazacort treatment (these are the LTAs and DIA muscles from the same animals). This means that deflazacort significantly improves the status of muscles in mdx mice, by sparing them from damage, which therefore reduces the requirement for repair, and reduces CNI as a result. DIA also shows a significantly lower CNI after deflazacort, but the decrease is much less than for LTA deflazacort vs. placebo.

L-NAME treatment (L-N) was then added to deflazacort to see if part of the effect of deflazacort was mediated by NO. The animals were given L-NAME in drinking water, at the same time as they got daily deflazacort injections, both over the 4 week treatment time. In these animals, the LTA CNI was no different than with deflazacort alone, which means the muscles with the less severe dystrophy were not affected by L-NAME treatment in combination with the full beneficial effect of deflazacort to reduce CNI. The DIA CNI was also not affected

when deflazacort was given with L-NAME, which suggests that the DIA has more severe dystrophy and combining deflazacort and an inhibitor of NOS activity to depress NOS activity to a lower level than it already is does not increase that severity.

The addition of the NO donor to deflazacort (D +L-Arginine) caused an increase in LTA CNI from deflazacort alone; (though CNI in deflazacort treatment plus L-Arginine is still significantly lower than placebo treatment). However, the NO donor did decrease the CNI of DIA from the level seen with D+L-NAME (i.e. it increased the benefit of deflazacort treatment in the diaphragm). The difference between the D+L-N effect on LTA and DIA suggests that the *in situ* treatment paradigm for applying NO manipulation in muscle repair is required to optimize its effects, and also that it could be used to augment the effects of steroids like deflazacort. This demonstrates that manipulating NO-mediated activation by changing NOS activity can be most useful when applied in situ to muscles in vivo, since systemic effects can benefit one muscle type (one phenotype of dystrophy) differently (more or less) than in another muscle phenotype.

In summary, deflazacort did significantly reduce the CNI in both the LTA and diaphragm (DIA). The effect was counteracted by L-Arginine in LTA and increased by L-Arginine in DIA, indicating that the systemic effects of L-Arginine (e.g. on the vasculature) augmented the local effects on satellite cell activation. The effedct was counteracted by deflazacort in diaphragm, presumably because the persistent unregulated activation of satellite cells in mdx dystrophic muscle ("standby" mode) is reduced there by L-Arginine. As the mdx diaphragm is the mdx muscle with the most similar phenotype to DMD, this result shows that L-Arginine or other NO donors can augment the beneficial effects of a steroid such as deflazacort, especially if given locally.

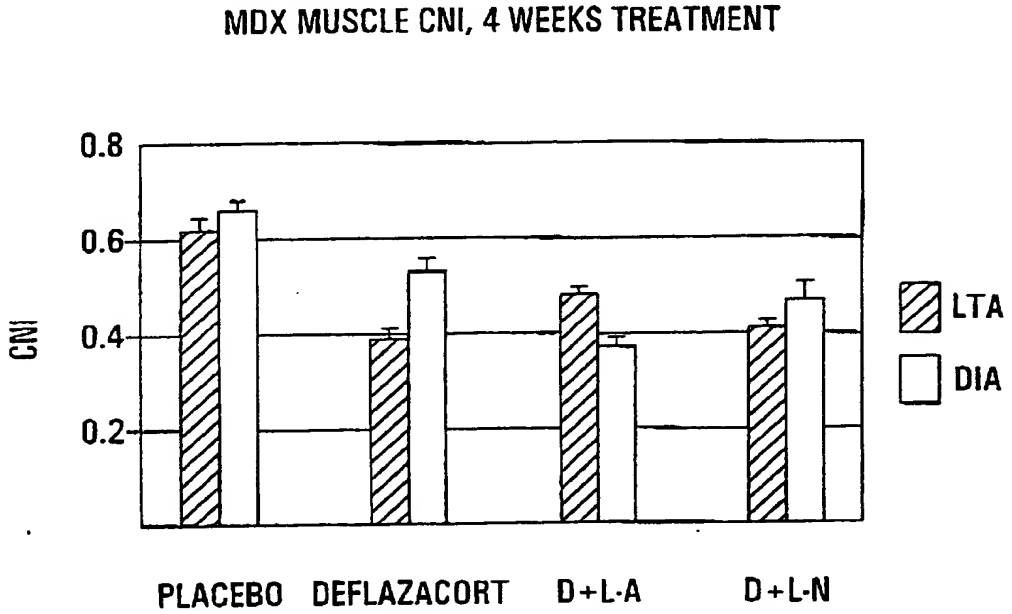
MDX MUSCLE CNI, 4 WEEKS TREATMENT
0.8
0.6
0.4
0.2
CNI
LTA
DIA
PLACEBO
DEFLAZACORT
D+L-A
D+L-N

FIG. 14

SPECIFICATION

MODULATION OF SKELETAL MUSCLE PRECURSOR CELL ACTIVATION

FIELD OF INVENTION

The present invention relates generally to skeletal muscle proliferation. More specifically, the invention relates to nitric oxide as a modulator of skeletal muscle precursor cell activation, and to uses of nitric oxide to improve muscle formation and repair in normal and disease states.

BACKGROUND OF THE INVENTION

Skeletal muscle arises after the induction of the mesoderm. After differentiation of the mesoderm into dorsal, intermediate, and lateral mesoderm, the dorsal mesodermal mesenchyme differentiates to form myotomes which, in turn, differentiate to give rise to the myogenic precursor cells which ultimately form skeletal muscle. Unlike the myogenic precursor cells of the heart, the skeletal muscle precursors fuse side-to-side to form unbranched, multinucleated myofibers. Some of the skeletal myogenic precursor cells do not differentiate and fuse into myocytes (also called myofibers) but, rather, attach to the outside of the plasmalemma of the myocytes. These cells participate in muscle growth during maturation and typically thereafter will remain, throughout adulthood, as largely undifferentiated, quiescent skeletal muscle "satellite cells." Upon injury of a skeletal muscle, these satellite cells are revealed to be myogenic precursor cells, or muscle "stem cells," which proliferate and differentiate, again by fusion, into new and functional skeletal muscle. Even after injury, some of the proliferated satellite cells remain undifferentiated and attach to the newly

formed myofibers. Thus, the satellite cells of skeletal muscle provide a constant and renewable source of myogenic precursor cells which allows for skeletal muscle repair and regeneration throughout mammalian life.

5

Exp. Cell Res. 216: 325-334; Anderson, J.E. et al. (1998) Muscle Nerve 21: 1153-1165; Floss, T., Arnold, H.-H., and Braun, T., (1997) Genes Dev. 11: 2040-2051). The timing and sequence of events are specific to repair (Megeney, L.A., Kablar, B., Garrett, K., Anderson J.E., and Rudnicki, M.A., (1996) Genes Dev. 10: 1173-1183; Li, Z., Mericskay, M., Agbulut, O., Butler-Browne, G. Carlsson, L., Thronell, L. E., Babinet, C., and Paulin, D., (1997) J. Cell Biol. 139: 129-144; McIntosh, L.M., Garrett, K.L., Megeney L., Rudnicki, M.A., and Anderson, J.E., (1998b) Anat. Rec. 252: 311-324) although similar to development (Rudnicki, M.A., and Jaenisch, R., (1995) Bioessays 17: 203-209; Yun, K., and Wold, B. (1996) Current Opinion Cell Biol. 8: 877-889).

The fine structure of satellite cells, positioned intimately between the fiber sarcolemma and external lamina (Mauro, A. (1961) J. Biophys. Biochem. Cytol. 87: 225-251; Ishikawa, H. (1966) Z. Anat. Entwicklungsgesch 125: 43-63) changes during their transition from quiescence to activation. Nuclei enlarge and become euchromatic. The typical attenuated organelle-poor cytoplasm expands and organelles such as mitochondria and rough endoplasmic reticulum hypertrophy (Schultz (1976) Am. J. Anat. 147: 49-70; Snow (1977) Cell Tissue Res. 185, 399-408; Schultz et al. (1978) J. Exp. Zool. 206: 451-456; Schultz et al. (1985) Muscle Nerve 8: 217-222). However, while activation is recognised as essential to repair and defined as precursor stimulation and recruitment to cycle (Bischoff, R. (1990a). J. Cell Biol. 111: 201-207), the initial signal, timing and character of activation are not known (Schultz and McCormick (1994) Rev. Physiol Biochem. Pharmacol. 123: 213-257).

To date, the earliest indicator of satellite cell transformation during activation is the co-localization of hepatocyte growth factor (also called scatter factor, HGF/SF) with its receptor c-met shortly after injury in normal rat muscle (Tatsumi et al. (1998) Dev. Biol. 194: 114-128). In

polyamino acids that do not possess an ascertained biological function, and derivatives thereof), S-nitrosylated amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof), S-nitrosated sugars, S-nitrosated oligonucleotides and derivatives thereof, S-nitrosated hydrocarbons where the hydrocarbon can be a branched or unbranched, saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon, S-nitroso hydrocarbons having one or more substituent groups in addition to the S-nitroso group, and heterocyclic compounds. S-nitrosothiols and the methods for preparing them are described in U.S. Pat. No. 5,380,758, filed Sep. 14, 1992; Oae et al. (1983) Org. Prep. Proc. Int. 15(3): 165-198; Loscalzo et al. (1989) J. Pharmacol. Exp. Ther. 249(3): 726-729 and Kowaluk et al. (1990) J. Pharmacol. Exp. Ther. 256: 1256-1264.

III. INHIBITORS OF NO ACTIVITY:

Inhibitors of NO activity (NO inhibitors) contemplated for use in the invention are compounds which chemically reacts with NO, binds to NO, or otherwise interacting with NO in such a way that the effective concentration of NO is reduced. Such inhibitors of NO activity include, but are not limited to, NO scavengers such as membrane impermeable NO scavengers including MGD-FE (N-methyl-D-glucamine dithiocarbamate/ferrous sulfate mixture), carboxy PTIO (2-(4-carboxyphenyl) 4,4,5,5-tetra methylimidazoline-1-oxyl 3-oxide), calcium chelator BAPTA/AM, S-nitroso-N-acetylpenicillamine (SNAP), 3-morpholino sydnonimine (SIN-1), diethyldithiocarbamate, melatonin and its precursors, superoxide dismutase, glutathione peroxidase, glutathione reductase, dimethyl sufoxide.

IV. REGULATORS OF NITRIC OXIDE PRODUCTION:

As an alternative to NO and NO donors, regulators of NO production may be used in the practice of the present

CLAIMS:

1. Use of NO, an NO donor, an NO inhibitor or a regulator of NO production, to modulate activation of skeletal muscle satellite cells.

2. Use of NO, an NO donor, or a regulator of NO production, to increase activation of skeletal muscle satellite cells.

3. Use of NO, an NO donor, or a regulator of NO production, to initiate new muscle formation.

4. The use according to claim 2 or 3, wherein the increase is localized to a limited area.

5. The use according to claim 2 or 3, wherein the increase is systemic.

6. Use of an NO inhibitor or a regulator of NO production to decrease activation of skeletal muscle satellite cells.

7. Use of an NO inhibitor or a regulator of NO production to decrease proliferation of muscle cells from skeletal muscle satellite cells.

8. The use according to claim 6 or 7, wherein the decrease is localized to a limited area.

9. The use according to claim 6 or 7, wherein the decrease is systemic.

10. Use of NO, an NO donor, an NO inhibitor or a regulator of NO production to modulate the effects of steroid hormone on skeletal muscle.

11. A method for modulating activation of a skeletal muscle satellite cell comprising contacting a muscle fiber containing the cell with an agent selected from the group consisting of NO, an NO donor, an NO inhibitor and a regulator of NO production.

12. A method for increasing activation of a skeletal muscle satellite cell comprising contacting a muscle fiber containing the cell with an agent selected from the group consisting of NO, an NO donor and a regulator of NO production.

13. A method for initiating new muscle formation comprising contacting a muscle fiber containing skeletal muscle satellite cells with an agent selected from the group consisting of NO, an NO donor and a regulator of NO production.

14. The method according to claim 12 or 13, wherein the increase is localized to a limited area.

15. The method according to claim 12 or 13, wherein the increase is systemic.

16. A method for activating a skeletal muscle satellite cell comprising contacting a muscle fiber containing the cell with an NO inhibitor or a regulator of NO production.

17. A method for decreasing proliferation of muscle cells from a skeletal muscle satellite cell comprising contacting a muscle fiber containing the satellite cell with an NO inhibitor or a regulator of NO production.

18. The method according to claim 16 or 17, wherein the decrease is localized to a limited area.

19. The method according to claim 16 or 17, wherein the decrease is systemic.

20. A method for modulating effects of steroid hormone on muscle comprising contacting a muscle fiber containing skeletal muscle satellite cells with an agent selected from the group consisting of NO, an NO donor, an NO inhibitor and a regulator of NO production in the presence of steroid hormone.

21. A method for amplifying muscle cells in culture, comprising contacting a muscle fiber containing skeletal muscle satellite cells with an agent selected from the group consisting of NO, an NO donor, and a regulator of NO production.

22. A method for obtaining a muscle cell population in culture, comprising contacting a muscle fiber containing skeletal muscle satellite cells in culture with an agent selected from the group consisting of NO, an NO donor, and a regulator of NO production.

23. A composition comprising skeletal muscle satellite cells and a compound selected from the group consisting of NO, an NO donor and a regulator of NO production.

24. A composition comprising any one of the group consisting of NO, an NO donor, an NO inhibitor and a regulator of NO production, and a diluent or carrier suitable for use in muscle, for modulating activation of skeletal muscle satellite cells.

25. A composition comprising a compound selected from the group consisting of NO, an NO donor, an NO inhibitor and a regulator of NO production, and a component suitable for increasing concentration of the compound in skeletal muscle, for modulating activation of skeletal muscle satellite cells.

26. The composition according to claim 25 wherein the component is a muscle-targeting component.

27. The composition according to claim 26 wherein the muscle-targeting component is an antibody or an antibody fragment with binding specificity against a protein selected from the group consisting of Bcl-2, HGF, M-cadherin, HGF-activating enzyme and collagen IV.

28. A commercial package containing as an active ingredient an agent selected from the group consisting of NO, an NO donor, an NO inhibitor and a regulator of NO production, together with instructions for its use for modulating activation of skeletal muscle satellite cells.

29. A commercial package containing as an active ingredient the composition of any one of claims 23 to 27, together with instructions for its use for modulating activation of skeletal muscle satellite cells.

30. The use according to any one of claims 1 to 5 and 10 wherein the NO donor is selected from the group consisting of organic nitrates, organic nitrites, inorganic nitroso compounds, sydnonimines, furoxans and S-nitrosothiols.

31. The method according to any one of claims 11 to 15 and 20 to 22 wherein the NO donor is selected from the group consisting of organic nitrates, organic nitrites, inorganic nitroso compounds, sydnonimines, furoxans and S-nitrosothiols.

32. The composition according to any one of claims 23 to 27 wherein the NO donor is selected from the group consisting of organic nitrates, organic nitrites, inorganic nitroso compounds, sydnonimines, furoxans and S-nitrosothiols.

33. The package according to claim 28 or 29 wherein the NO donor is selected from the group consisting of organic nitrates, organic nitrites, inorganic nitroso compounds, sydnonimines, furoxans and S-nitrosothiols.

34. The use according to any one of claims 1 to 5 and 10 wherein the NO donor is L-arginine.

35. The method according to any one of claims 11 to 15, 20 to 22 wherein the NO donor is L-arginine.

36. The composition according to any one of claims 23 to 27 wherein the NO donor is L-arginine.

37. The package according to claim 28 or 29 wherein the NO donor is L-arginine.

38. The use according to any one of claims 1 and 6 to 10 wherein the NO inhibitor is selected from the group consisting of N-methyl-D-glucamine dithiocarbamate/ferrous sulfate mixture, carboxy PTIO (2-(4-carboxyphenyl) 4,4,5,5-tetra methylimidazoline-1-oxyl 3-oxide), calcium chelator BAPTA/AM, S-nitroso-N-acetylpenicillamine, 3-morpholino sydnonimine, diethyldithiocarbamate, melatonin and its precursors, superoxide dismutase, glutathione peroxidase, glutathione reductase, and dimethyl sufoxide.

39. The method according to any one of claims 11 and 16 to 20 wherein the NO inhibitor is selected from the group consisting of N-methyl-D-glucamine dithiocarbamate/ferrous sulfate mixture, carboxy PTIO (2-(4-carboxyphenyl) 4,4,5,5-tetra methylimidazoline-1-oxyl 3-oxide), calcium chelator BAPTA/AM, S-nitroso-N-acetylpenicillamine, 3-morpholino sydnonimine, diethyldithiocarbamate, melatonin and its precursors, superoxide dismutase, glutathione peroxidase, glutathione reductase, and dimethyl sufoxide.

40. The composition according to any one of claims 24 to 27 wherein the NO inhibitor is selected from the group consisting of N-methyl-D-glucamine dithiocarbamate/ferrous sulfate mixture, carboxy PTIO (2-(4-carboxyphenyl) 4,4,5,5-

tetra methylimidazoline-1-oxyl 3-oxide), calcium chelator BAPTA/AM, S-nitroso-N-acetylpenicillamine, 3-morpholino sydnonimine, diethyldithiocarbamate, melatonin and its precursors, superoxide dismutase, glutathione peroxidase, glutathione reductase, and dimethyl sufoxide.

41. The package according to claim 28 or 29 wherein the NO inhibitor is selected from the group consisting of N-methyl-D-glucamine dithiocarbamate/ferrous sulfate mixture, carboxy PTIO (2-(4-carboxyphenyl) 4,4,5,5-tetra methylimidazoline-1-oxyl 3-oxide), calcium chelator BAPTA/AM, S-nitroso-N-acetylpenicillamine, 3-morpholino sydnonimine, diethyldithiocarbamate, melatonin and its precursors, superoxide dismutase, glutathione peroxidase, glutathione reductase, and dimethyl sufoxide.

42. The use according to any one of claims 1 to 10 wherein the regulator of NO production is selected from the group consisting of nitric oxide synthase (NOS), enhancer of NOS activity, inhibitor of NOS activity, enhancer of NOS gene expression and a variant of NOS.

43. The method according to any one of claims 11 to 22 wherein the regulator of NO production is selected from the group consisting of nitric oxide synthase (NOS), enhancer of NOS activity, inhibitor of NOS activity, enhancer of NOS gene expression and a variant of NOS.

44. The composition according to any one of claims 23 to 27 wherein the regulator of NO production is selected from the group consisting of nitric oxide synthase (NOS), enhancer of NOS activity, inhibitor of NOS activity, enhancer of NOS gene expression and a variant of NOS.

45. The package according to claim 28 or 29 wherein the regulator of NO production is selected from the group consisting of nitric oxide synthase (NOS), enhancer of NOS activity, inhibitor of NOS activity, enhancer of NOS gene expression and a variant of NOS.

46. The use according to any one of claims 1 to 10 wherein the regulator of NO production is nitric oxide synthase NOS 1μ.

47. The method according to any one of claims 11 to 22 wherein the regulator of NO production is nitric oxide synthase NOS 1μ.

48. The composition according to any one of claims 23 to 27 wherein the regulator of NO production is nitric oxide synthase NOS 1μ.

49. The package according to claim 28 or 29 wherein the regulator of NO production is nitric oxide synthase NOS 1μ.

50. The use according to claim 42 wherein the inhibitor of NOS activity is Nω-nitro-L-arginine methyl ester (L-NAME).

51. The method according to claim 43 wherein the inhibitor of NOS activity is Nω-nitro-L-arginine methyl ester (L-NAME).

52. The composition according to claim 44 wherein the inhibitor of NOS activity is Nω-nitro-L-arginine methyl ester (L-NAME).

53. The package according to claim 45 wherein the inhibitor of NOS activity is Nω-nitro-L-arginine methyl ester (L-NAME).

54. The use according to claim 10 wherein the steroid hormone is an anabolic steroid or a glucocorticoid.

55. The use according to claim 54 wherein the steroid hormone is selected from the group consisting of deflazacort, a derivative of prednisone or a derivative of methyl-prednisone.

56. The use according to claim 54 wherein the steroid hormone is deflazacort.

57. The method according to claim 20 wherein the steroid hormone is an anabolic steroid or a glucocorticoid.

58. The method according to claim 57 wherein the steroid hormone is selected from the group consisting of deflazacort, a derivative of prednisone or a derivative of methyl-prednisone.

59. The method according to claim 57 wherein the steroid hormone is deflazacort.

60. Use for modulating skeletal muscle satellite cells in a muscle fiber of a vertebrate animal, of a substance selected from the group consisting of NO, an NO donor, an NO inhibitor, a regulator of NO production, and the composition according to any one of claims 23 to 27.

61. A method for modulating skeletal muscle satellite cells in a muscle fiber of a vertebrate animal, comprising contacting the muscle fiber with a substance selected from the group consisting of NO, an NO donor, an NO inhibitor, a regulator of NO production, and the composition according to any one of claims 23 to 27.

62. Use for regenerating muscle tissue from skeletal muscle satellite cells in human dystrophy, of a substance selected from the group consisting of NO, an NO donor, an NO

inhibitor, a regulator of NO production, and the composition according to any one of claims 23 to 27.

63. A method for regenerating muscle tissue from skeletal muscle satellite cells of a muscle fiber in human dystrophy, comprising contacting the muscle fiber with a substance selected from the group consisting of NO, an NO donor, an NO inhibitor, a regulator of NO production, and the composition according to any one of claims 23 to 27.

64. Use for augmenting steroid hormone treatment of human muscle dystrophy, of a substance selected from the group consisting of NO, an NO donor, an NO inhibitor, a regulator of NO production, and the composition according to any one of claims 23 to 27, in conbination with steroid hormone.

65. A method for augmenting steroid hormone treatment of human dystrophy, comprising contacting muscle tissue with a substance selected from the group consisting of NO, an NO donor, an NO inhibitor, a regulator of NO production, and the composition according to any one of claims 23 to 27, in the presence of steroid hormone.

66. The use according to claim 62 wherein the dystrophy is selected from the group consisting of Duchenne, Becker, Emery-Dreifuss, Landouzy-Dejerine, Scapulohumeral of Seitz, Limb-girdle (Erb), von Graefe-Fuchs, Oculopharyngeal, Myotonic (Steinert) and Congenital dystrophy.

67. The method according to claim 63 wherein the dystrophy is selected from the group consisting of Duchenne, Becker, Emery-Dreifuss, Landouzy-Dejerine, Scapulohumeral of Seitz, Limb-girdle (Erb), von Graefe-Fuchs, Oculopharyngeal, Myotonic (Steinert) and Congenital dystrophy.

68. A method for validating a test wherein a change in activation state of muscle precursor cells is determined, comprising use of a DNA intercalator to determine that fibers associated with the precursor cells are intact.

69. A method for validating a test wherein a fiber hypercontraction-dependent change in activation state of muscle precursor cells is determined, comprising use of a myotoxin and a DNA intercalator to determine fiber membrane damage.

70. The method according to claim 68 or 69 wherein the test is a diagnostic test.

71. A method for identifying a compound which effects a change in activation state of skeletal muscle satellite cells, comprising:

a) determining that fibers associated with the satellite cells are intact;

b) determining the activation state of satellite cells in the absence of the compound; and

c) determining the activation state of satellite cells treated with the compound;

wherein the difference between the two activation states identify the compound as a compound which effects a change in activation state of skeletal muscle satellite cells.

72. A method for identifying a compound which effects a fiber hypercontraction-dependent change in activation state of skeletal muscle satellite cells, comprising:

a) treating an intact fiber containing skeletal muscle satellite cells with a myotoxin and a DNA intercalator to effect fiber hypercontraction;

b) determining the activation state of skeletal muscle satellite cells in the absence of the myotoxin, DNA intercalator and the compound; and

c) determining the activation state of skeletal muscle satellite cells treated with the compound in the absence of the myotoxin and DNA intercalator;

wherein the difference between the two activation states identify the compound as a compound which effects a fiber hypercontraction-dependent change in activation state of skeletal muscle satellite cells.

73. The method according to any one of claims 68 to 70 and 72 wherein the DNA intercalator is ethidium bromide or propidium iodide.

74. The method according to claim 69 or 72 wherein the myotoxin is marcaine.

75. The in vitro use according to any one of claims 1 to 10, 30, 34, 38, 42, 46, 50, 54 to 56 and 66.

76. The in vitro method according to any one of claims 11 to 22, 31, 35, 39, 43, 47, 51, 57 to 59, 61, 63, 65 and 67 to 74.

77. The commercial package according to any one of claims 28, 29, 33, 37, 41, 45, 49 and 53 for use in vitro.

SMART & BIGGAR

OTTAWA, CANADA

PATENT AGENTS

SPECIFICATION

NITRIC OXIDE MANIPULATION OF MUSCLE SATELLITE CELL ACTIVATION

FIELD OF INVENTION

The present invention relates generally to skeletal muscle proliferation. More specifically, the invention relates to nitric oxide as a modulator of skeletal muscle precursor cell activation, and to uses of nitric oxide to improve muscle formation and repair in normal and disease states.

BACKGROUND OF THE INVENTION

Skeletal muscle arises after the induction of the mesoderm. After differentiation of the mesoderm into dorsal, intermediate, and lateral mesoderm, the dorsal mesodermal mesenchyme differentiates to form myotomes which, in turn, differentiate to give rise to the myogenic precursor cells which ultimately form skeletal muscle. Unlike the myogenic precursor cells of the heart, the skeletal muscle precursors fuse side-to-side to form unbranched, multinucleated myofibers. Some of the skeletal myogenic precursor cells do not differentiate and fuse into myocytes (also called myofibers) but, rather, attach to the outside of the plasmalemma of the myocytes. These cells participate in muscle growth during maturation and typically thereafter will remain, throughout adulthood, as largely undifferentiated, quiescent skeletal muscle "satellite cells." Upon injury of a skeletal muscle, these satellite cells are revealed to be myogenic precursor cells, or muscle "stem cells," which proliferate and differentiate, again by fusion, into new and functional skeletal muscle. Even after injury, some of the proliferated satellite cells remain undifferentiated and attach to the newly

CLAIMS:

1. Use of an agent selected from the group consisting of: NO, an NO donor, an NO inhibitor or a regulator of NO production, to modulate and sustain skeletal muscle formation and/or repair.

2. The use according to claim 1, wherein the agent is NO, an NO donor, or a regulator of NO production, to increase and sustain skeletal muscle formation and/or repair.

3. The use according to claim 2, to initiate and sustain skeletal muscle formation and/or repair.

4. The use according to claim 2 or 3, wherein the increase is localized to a limited area.

5. The use according to claim 2 or 3, wherein the increase is systemic.

6. Use according to claim 1 of an NO inhibitor or a down-regulator of NO production to decrease activation of skeletal muscle satellite cells, thereby treating muscular dystrophy.

7. The use according to claim 6 to decrease proliferation of skeletal muscle cells.

8. The use according to claim 6 or 7, wherein the decrease is localized to a limited area.

9. The use according to claim 6 or 7, wherein the decrease is systemic.

10. Use of an agent selected from the group consisting of: NO, an NO donor, an NO inhibitor or a regulator of NO

production, to modulate the effects of steroid hormone on skeletal muscle.

11. A method for modulating and sustaining skeletal muscle formation and/or repair, comprising contacting a skeletal muscle fiber containing satellite cells with an agent selected from the group consisting of NO, an NO donor, an NO inhibitor and a regulator of NO production.

12. The method according to claim 11 for increasing and sustaining skeletal muscle formation and/or repair, wherein the agent is selected from the group consisting of NO, an NO donor and an up-regulator of NO production.

13. The method according to claim 12 for initiating and sustaining skeletal muscle formation and/or repair.

14. The method according to claim 12 or 13, wherein the increase is localized to a limited area.

15. The method according to claim 12 or 13, wherein the increase is systemic.

16. The method according to claim 12 wherein the agent is an NO donor or or an up-regulator of NO production.

17. The method according to claim 11 for decreasing proliferation of skeletal muscle cells, wherein the agent is an NO inhibitor or a down-regulator of NO production.

18. The method according to claim 16 or 17, wherein the decrease is localized to a limited area.

19. The method according to claim 16 or 17, wherein the decrease is systemic.

20. A method for modulating effects of steroid hormone on skeletal muscle comprising contacting a muscle fiber containing skeletal muscle satellite cells with an agent selected from the group consisting of NO, an NO donor, an NO inhibitor and a regulator of NO production, in the presence of steroid hormone.

21. A method for amplifying muscle cells in culture, comprising contacting a muscle fiber containing skeletal muscle satellite cells with an agent selected from the group consisting of NO, an NO donor, and an up-regulator of NO production.

22. The method according to claim 21 for obtaining a muscle cell population in culture.

23. A composition comprising skeletal muscle satellite cells and an agent selected from the group consisting of NO, an NO donor and a regulator of NO production.

24. A composition comprising an agent selected from the group consisting of NO, an NO donor, an NO inhibitor and a regulator of NO production, and a diluent or carrier suitable for use in skeletal muscle, for modulating and sustaining skeletal muscle formation and/or repair.

25. The composition according to claim 24, further comprising a component suitable for increasing concentration of the agent in skeletal muscle.

26. The composition according to claim 25 wherein the component is a skeletal muscle-targeting component.

27. The composition according to claim 26 wherein the muscle-targeting component is an antibody or an antibody fragment with binding specificity against a protein selected

from the group consisting of Bcl-2, HGF, M-cadherin, HGF-activating enzyme and collagen IV.

28. A commercial package containing as an active ingredient an agent selected from the group consisting of NO, an NO donor, an NO inhibitor, a regulator of NO production, and the composition as defined in any one of claims 23 to 27, together with instructions for its use for modulating and sustaining skeletal muscle formation and/or repair.

29. The use according to any one of claims 1 to 5 and 10 wherein the NO donor is selected from the group consisting of organic nitrates, organic nitrites, inorganic nitroso compounds, sydnonimines, furoxans and S-nitrosothiols.

30. The method according to any one of claims 11 to 15 and 20 to 22 wherein the NO donor is selected from the group consisting of organic nitrates, organic nitrites, inorganic nitroso compounds, sydnonimines, furoxans and S-nitrosothiols.

31. The composition according to any one of claims 23 to 27 wherein the NO donor is selected from the group consisting of organic nitrates, organic nitrites, inorganic nitroso compounds, sydnonimines, furoxans and S-nitrosothiols.

32. The package according to claim 28 wherein the NO donor is selected from the group consisting of organic nitrates, organic nitrites, inorganic nitroso compounds, sydnonimines, furoxans and S-nitrosothiols.

33. The use according to any one of claims 1 to 5 and 10 wherein the NO donor is L-arginine.

34. The method according to any one of claims 11 to 15, 20 to 22 wherein the NO donor is L-arginine.

35. The composition according to any one of claims 23 to 27 wherein the NO donor is L-arginine.

36. The package according to claim 28 wherein the NO donor is L-arginine.

37. The use according to any one of claims 1 and 6 to 10 wherein the NO inhibitor is selected from the group consisting of N-methyl-D-glucamine dithiocarbamate/ferrous sulfate mixture, carboxy PTIO (2-(4-carboxyphenyl) 4,4,5,5-tetra methylimidazoline-1-oxyl 3-oxide), calcium chelator BAPTA/AM, S-nitroso-N-acetylpenicillamine, 3-morpholino sydnonimine, diethyldithiocarbamate, melatonin and its precursors, superoxide dismutase, glutathione peroxidase, glutathione reductase, and dimethyl sulfoxide.

38. The method according to any one of claims 11 and 16 to 20 wherein the NO inhibitor is selected from the group consisting of N-methyl-D-glucamine dithiocarbamate/ferrous sulfate mixture, carboxy PTIO (2-(4-carboxyphenyl) 4,4,5,5-tetra methylimidazoline-1-oxyl 3-oxide), calcium chelator BAPTA/AM, S-nitroso-N-acetylpenicillamine, 3-morpholino sydnonimine, diethyldithiocarbamate, melatonin and its precursors, superoxide dismutase, glutathione peroxidase, glutathione reductase, and dimethyl sulfoxide.

39. The composition according to any one of claims 24 to 27 wherein the NO inhibitor is selected from the group consisting of N-methyl-D-glucamine dithiocarbamate/ferrous sulfate mixture, carboxy PTIO (2-(4-carboxyphenyl) 4,4,5,5-tetra methylimidazoline-1-oxyl 3-oxide), calcium chelator BAPTA/AM, S-nitroso-N-acetylpenicillamine, 3-morpholino sydnonimine, diethyldithiocarbamate, melatonin and its precursors, superoxide dismutase, glutathione peroxidase, glutathione reductase, and dimethyl sulfoxide.

40. The package according to claim 28 wherein the NO inhibitor is selected from the group consisting of N-methyl-D-glucamine dithiocarbamate/ferrous sulfate mixture, carboxy PTIO (2-(4-carboxyphenyl) 4,4,5,5-tetra methylimidazoline-1-oxyl 3-oxide), calcium chelator BAPTA/AM, S-nitroso-N-acetylpenicillamine, 3-morpholino sydnonimine, diethyldithiocarbamate, melatonin and its precursors, superoxide dismutase, glutathione peroxidase, glutathione reductase, and dimethyl sulfoxide.

41. The use according to any one of claims 1 to 10 wherein the regulator of NO production is selected from the group consisting of nitric oxide synthase (NOS), enhancer of NOS activity, inhibitor of NOS activity, enhancer of NOS gene expression and a variant of NOS.

42. The method according to any one of claims 11 to 22 wherein the regulator of NO production is selected from the group consisting of nitric oxide synthase (NOS), enhancer of NOS activity, inhibitor of NOS activity, enhancer of NOS gene expression and a variant of NOS.

43. The composition according to any one of claims 23 to 27 wherein the regulator of NO production is selected from the group consisting of nitric oxide synthase (NOS), enhancer of NOS activity, inhibitor of NOS activity, enhancer of NOS gene expression and a variant of NOS.

44. The package according to claim 28 wherein the regulator of NO production is selected from the group consisting of nitric oxide synthase (NOS), enhancer of NOS activity, inhibitor of NOS activity, enhancer of NOS gene expression and a variant of NOS.

45. The use according to any one of claims 1 to 10 wherein the regulator of NO production is nitric oxide synthase NOS 1μ.

46. The method according to any one of claims 11 to 22 wherein the regulator of NO production is nitric oxide synthase NOS 1μ.

47. The composition according to any one of claims 23 to 27 wherein the regulator of NO production is nitric oxide synthase NOS 1μ.

48. The package according to claim 28 wherein the regulator of NO production is nitric oxide synthase NOS 1μ.

49. The use according to claim 41 wherein the inhibitor of NOS activity is Nω-nitro-L-arginine methyl ester (L-NAME).

50. The method according to claim 42 wherein the inhibitor of NOS activity is Nω-nitro-L-arginine methyl ester (L-NAME).

51. The composition according to claim 43 wherein the inhibitor of NOS activity is Nω-nitro-L-arginine methyl ester (L-NAME).

52. The package according to claim 44 wherein the inhibitor of NOS activity is Nω-nitro-L-arginine methyl ester (L-NAME).

53. The use according to claim 10 wherein the steroid hormone is an anabolic steroid or a glucocorticoid.

54. The use according to claim 53 wherein the steroid hormone is selected from the group consisting of deflazacort, a derivative of prednisone or a derivative of methyl-prednisone.

55. The use according to claim 53 wherein the steroid hormone is deflazacort.

56. The method according to claim 20 wherein the steroid hormone is an anabolic steroid or a glucocorticoid.

57. The method according to claim 56 wherein the steroid hormone is selected from the group consisting of deflazacort, a derivative of prednisone or a derivative of methyl-prednisone.

58. The method according to claim 56 wherein the steroid hormone is deflazacort.

59. The use according to any one of claims 1 to 10, to modulate and sustain skeletal muscle formation and/or repair of a vertebrate animal.

60. The method according to any one of claims 11 to 20, for modulating and sustaining skeletal muscle formation and/or repair of a vertebrate animal.

61. The use according to any one of claims 1 to 10, for regenerating skeletal muscle tissue in human dystrophy.

62. The method according to any one of claims 11 to 20, for regenerating skeletal muscle tissue in human dystrophy.

63. The use according to claim 10 for augmenting steroid hormone treatment of human muscle dystrophy, wherein the agent is used in combination with steroid hormone.

64. The method according to claim 20 for augmenting steroid hormone treatment of human dystrophy, wherein the agent contacts the muscle fiber in the presence of steroid hormone.

65. The use according to claim 61 wherein the dystrophy is selected from the group consisting of Duchenne, Becker,

Emery-Dreifuss, Landouzy-Dejerine, Scapulohumeral of Seitz, Limb-girdle (Erb), von Graefe-Fuchs, Oculopharyngeal, Myotonic (Steinert) and Congenital dystrophy.

66. The method according to claim 62 wherein the dystrophy is selected from the group consisting of Duchenne, Becker, Emery-Dreifuss, Landouzy-Dejerine, Scapulohumeral of Seitz, Limb-girdle (Erb), von Graefe-Fuchs, Oculopharyngeal, Myotonic (Steinert) and Congenital dystrophy.

67. A method for validating a test wherein a change in activation state of muscle precursor cells is determined, comprising use of a DNA intercalator to determine that fibers associated with the precursor cells are intact.

68. The method according to claim 67 wherein the change in activation state is a fiber hypercontraction-dependent change, and wherein the DNA intercalator is used with a myotoxin to determine fiber membrane damage.

69. The method according to claim 67 or 68 wherein the test is a diagnostic test.

70. A method for identifying a compound which effects a change in activation state of skeletal muscle satellite cells, comprising:

a) determining that fibers associated with the satellite cells are intact;

b) determining the activation state of satellite cells in the absence of the compound; and

c) determining the activation state of satellite cells treated with the compound;

wherein the difference between the two activation states identify the compound as a compound which effects a change in activation state of skeletal muscle satellite cells.

71. A method for identifying a compound which effects a fiber hypercontraction-dependent change in activation state of skeletal muscle satellite cells, comprising:

a) treating an intact fiber containing skeletal muscle satellite cells with a myotoxin and a DNA intercalator to effect fiber hypercontraction;

b) determining the activation state of skeletal muscle satellite cells in the absence of the myotoxin, DNA intercalator and the compound; and

c) determining the activation state of skeletal muscle satellite cells treated with the compound in the absence of the myotoxin and DNA intercalator;

wherein the difference between the two activation states identify the compound as a compound which effects a fiber hypercontraction-dependent change in activation state of skeletal muscle satellite cells.

72. The method according to any one of claims 67 to 69 and 77 wherein the DNA intercalator is ethidium bromide or propidium iodide.

73. The method according to claim 68 or 71 wherein the myotoxin is marcaine.

74. The in vitro use according to any one of claims 1 to 10, 29, 31, 37, 41, 45, 49, 53 to 55 and 65.

75. The in vitro method according to any one of claims 11 to 22, 30, 34, 38, 42, 46, 50, 56 to 58, 60, 62, 64 and 66 to 73.

76. The commercial package according to any one of claims 28, 32, 36, 40, 44, 48 and 52 for use in vitro.

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 33/00

A2

(11) International Publication Number: WO 00/53191

(43) International Publication Date: 14 September 2000 (14.09.00)

(21) International Application Number: PCT/CA00/00255

(22) International Filing Date: 10 March 2000 (10.03.00)

(30) Priority Data:
60/123,895 11 March 1999 (11.03.99) US

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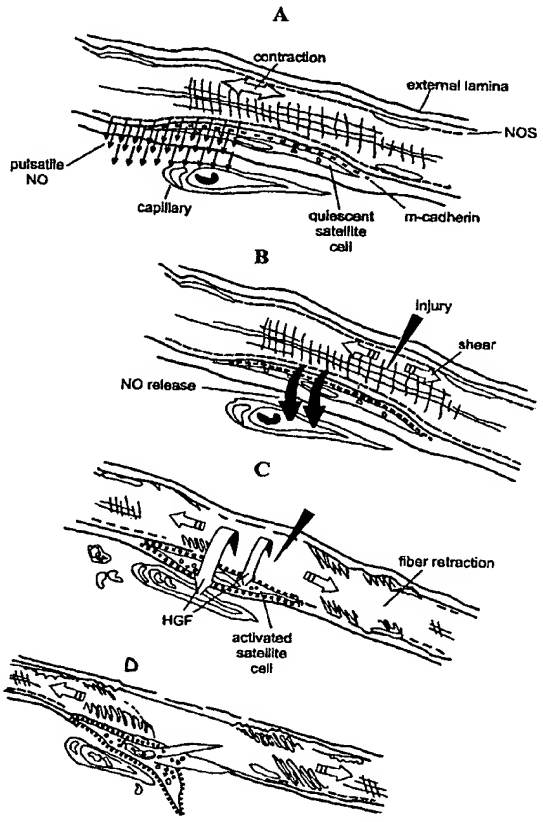
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: MODULATION OF SKELETAL MUSCLE PRECURSOR CELL ACTIVATION

(57) Abstract

The present invention is directed to methods, pharmaceutical compositions and kits for modulating skeletal muscle precursor cell activation. Modulation is effected through the use of nitric oxide (NO), donors of NO, inhibitors of NO activity (NO inhibitor) or regulators of NO production, either locally or systemically. The invention further teaches the use of NO, an NO donor, an NO inhibitor or a regulator of NO production to modulate the effects of steroid hormone on skeletal muscle. The invention further provides a method for identifying a compound which effects a change in activation state of muscle precursor cells. A number of advantages is evident. By allowing skeletal muscle precursor cells to be manipulated directly, the invention enables specific treatments to regenerate and repair muscle.



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TITLE OF INVENTION

MODULATION OF SKELETAL MUSCLE PRECURSOR CELL ACTIVATION

FIELD OF INVENTION

The present invention relates generally to skeletal muscle proliferation. More specifically, the invention relates to nitric oxide as a modulator of skeletal muscle precursor cell activation, and to uses of nitric oxide to improve muscle formation and repair in normal and disease states.

BACKGROUND OF THE INVENTION

Skeletal muscle arises after the induction of the mesoderm. After differentiation of the mesoderm into dorsal, intermediate, and lateral mesoderm, the dorsal mesodermal mesenchyme differentiates to form myotomes which, in turn, differentiate to form give rise to the myogenic precursor cells which ultimately form skeletal muscle. Unlike the myogenic precursor cells of the heart, the skeletal muscle precursors fuse side-to-side to form unbranched, multinucleated myofibers. Some of the skeletal myogenic precursor cells do not differentiate and fuse into myocytes (also called myofibers) but, rather, attach to the outside of the plasmalemma of the myocytes. These cells participate in muscle growth during maturation and typically thereafter will remain, throughout adulthood, as largely undifferentiated, quiescent skeletal muscle "satellite cells." Upon injury of a skeletal muscle, these satellite cells are revealed to be myogenic precursor cells, or muscle "stem cells," which proliferate and differentiate, again by fusion, into new and functional skeletal muscle. Even after injury, some of the proliferated satellite cells remain undifferentiated and attach to the newly formed myofibers. Thus, the satellite cells of skeletal muscle provide a constant and renewable source of myogenic precursor cells which allows for skeletal muscle repair and regeneration throughout mammalian life.

Exp. Cell Res. 216: 325-334; Anderson, J.E. et al. (1998) Muscle Nerve 21: 1153-1165; Floss, T., Arnold, H.-H., and Braun, T., (1997) Genes Dev. 11: 2040-2051). The timing and sequence of events are specific to repair (Megeney, L.A., Kablar, B., Garrett, K., Anderson J.E., and Rudnicki, M.A., (1996) Genes Dev. 10: 1173-1183; Li, Z., Mericskay, M., Agbulut, O., Butler-Browne, G. Carlsson, L., Thronell, L. E., Babinet, C., and Paulin, D., (1997) J. Cell Biol. 139: 129-144; McIntosh, L.M., Garrett, K.L., Megeney L., Rudnicki, M.A., and Anderson, J.E., (1998b) Anat. Rec. 252: 311-324) although similar to development (Rudnicki, M.A., and Jaenisch, R., (1995) Bioessays 17: 203-209; Yun, K., and Wold, B. (1996) Current Opinion Cell Biol. 8: 877-889).

The fine structure of satellite cells, positioned intimately between the fiber sarcolemma and external lamina (Mauro, A. (1961) J. Biophys. Biochem. Cytol. 87: 225-251; Ishikawa, H. (1966) Z. Anat. Entwicklungsgesch 125: 43-63) changes during their transition from quiescence to activation. Nuclei enlarge and become euchroamatic. The typical attenuated organelle-poor cytoplasm expands and organelles such as mitochondria and rough endoplasmic reticulum hypertrophy (Schultz (1976) Am. J. Anat. 147: 49-70; Snow (1977) Cell Tissue Res. 185, 399-408; Schultz et al. (1978) J. Exp. Zool. 206: 451-456; Schultz et al. (1985) Muscle Nerve 8: 217-222). However, while activation is recognised as essential to repair and defined as precursor stimulation and recruitment to cycle (Bischoff, R. (1990a). J. Cell Biol. 111: 201-207), the initial signal, timing and character of activation are not known (Schultz and McCormick (1994) Rev. Physiol Biochem. Pharmacol. 123: 213-257).

To date, the earliest indicator of satellite cell transformation during activation is the co-localization of hepatocyte growth factor (also called scatter factor, HGF/SF) with its receptor c-met shortly after injury in normal rat muscle (Tatsumi et al. (1998) Dev. Biol. 194: 114-128). In

polyamino acids that do not possess an ascertained biological function, and derivatives thereof), S-nitrosylated amino acids (including natural and synthetic amino acids and their stereoisomers and racemic mixtures and derivatives thereof), S-nitrosated sugars, S-nitrosated oligonucleotides and derivatives thereof, S-nitrosated hydrocarbons where the hydrocarbon can be a branched or unbranched, saturated or unsaturated aliphatic hydrocarbon, or an aromatic hydrocarbon, S-nitroso hydrocarbons having one or more substituent groups in addition to the S-nitroso group, and heterocyclic compounds. S-nitrosothiols and the methods for preparing them are described in U.S. Pat. No. 5,380,758, filed Sep. 14, 1992; Oae et al. (1983) Org. Prep. Proc. Int. 15(3): 165-198; Loscalzo et al. (1989) J. Pharmacol. Exp. Ther. 249(3): 726-729 and Kowaluk et al. (1990) J. Pharmacol. Exp. Ther. 256: 1256-1264.

III. INHIBITORS OF NO ACTIVITY:

Inhibitors of NO activity (NO inhibitors) contemplated for use in the invention are compounds which chemically reacts with NO, binds to NO, or otherwise interacting with NO in such a way that the effective concentration of NO is reduced. Such inhibitors of NO activity include, but are not limited to, NO scavengers such as membrane impermeable NO scavengers including MGD-FE (N-methosyl-D-glucamine dithiocarbamate/ferrous sulfate mixture), carboxy PTIO (2-(4-carboxyphenyl) 4,4,5,5-tetra methylimidazoline-1-oxyl 3-oxide), calcium chelator BAPTA/AM, S-nitroso-N-acetylpenicillamine (SNAP), 3-morpholini sydnonimine (SIN-1), diethyldithiocarbamate, melatonin and its precursors, superoxide dismutase, glutathione peroxidase, glutathione reductase, dimethyl sufoxide.

IV. REGULATORS OF NITRIC OXIDE PRODUCTION:

As an alternative to NO and NO donors, regulators of NO production may be used in the practice of the present

In this experiment, muscle tissues are collected from the treated animals. LTA and diaphragm muscles were sectioned, and counts of fibers with central nuclei and fibers with peripheral nuclei were performed. The largest diameter of the muscle section in each of the two sections of each muscle was used for counting, for 10 mice per group (2 muscles per mouse).

Figure 14 shows that in the mdx mouse, the CNI in placebo-treated animals is about 0.6 (i.e. 60% of fibers) show a centrally located nucleus in a cross section of the muscle. This is similar for the tibialis anterior muscle (LTA) and diaphragm at the age shown in the graph (which is 8 weeks of age) and is reliably used to monitor the progressive effect of dystrophic fiber injury on a muscle over time as the disease progresses. CNI will increase with age in the mdx mice (until the plateau discussed above). Mice are treated from 4-8 weeks of age with placebo, Deflazacort, D+L-NAME or D+L-Arginine.

With deflazacort treatment for 4 weeks, the CNI is significantly less than in placebo-treated mdx LTA (down to 0.4 or 40%) in the left TA (LTA). The CNI in diaphragm (DIA) also decreases with deflazacort treatment (these are the LTAs and DIA muscles from the same animals). This means that deflazacort significantly improves the status of muscles in mdx mice, by sparing them from damage, which therefore reduces the requirement for repair, and reduces CNI as a result. DIA also shows a significantly lower CNI after deflazacort, but the decrease is much less than for LTA deflazacort vs. placebo.

L-NAME treatment (L-N) was then added to deflazacort to see if part of the effect of deflazacort was mediated by NO. The animals were given L-NAME in drinking water, at the same time as they got daily deflazacort injections, both over the 4 week treatment time. In these animals, the LTA CNI was higher than with deflazacort alone, which means the muscles with the less severe dystrophy needed the activation to have the full beneficial effect of deflazacort to reduce CNI. By comparison, the DIA CNI was reduced further when deflazacort was given with

L-NAME, which suggests that the DIA has more severe dystrophy whose severity is reduced (and therefore CNI is lowered) by combining deflazacort and an inhibitor of NOS activity which would depress NOS activity to a lower level than it already is. The difference between the D+L-N effect on LTA and DIA suggests that the in situ treatment paradigm for applying NO manipulation in muscle repair is required to optimize its effects, and also that it could be used to augment the effects of steroids like deflazacort.

The addition of the NO donor to deflazacort (D +L-Arginine) caused no change in LTA CNI from deflazacort alone; (though CNI in deflazacort treatment alone is still significantly lower than placebo treatment). However, the NO donor did raise the CNI of DIA from the level seen with D+L-NAME (i.e. it negated the benefit of NOS inhibition in the diaphragm). This demonstrates that manipulating NO-mediated activation by changing NOS activity can be most useful when applied in situ to muscles in vivo, since systemic effects can benefit one muscle type (one phenotype of dystrophy) differently (more or less) than in another muscle phenotype.

In summary, deflazacort did significantly reduce the CNI in both the LTA and diaphragm (DIA). The effect was counteracted by L-NAME in LTA, indicating that the deleterious systemic effects of L-NAME (e.g. on the vasculature) prevailed over the local effects on satellite cell activation. However, it was clear that L-NAME augmented the beneficial effects of deflazacort in diaphragm, presumably because the persistent unregulated activation of satellite cells in mdx dystrophic muscle ("standby" mode) is reduced there. As the mdx diaphragm is the mdx muscle with the most similar phenotype to DMD, this result shows that L-NAME or other NOS inhibitors can augment the beneficial effects of a steroid such as deflazacort, especially if given locally.

CLAIMS:

1. Use of NO, an NO donor, an NO inhibitor or a regulator of NO production, to modulate activation of muscle precursor cells.

2. Use of NO, an NO donor, or a regulator of NO production, to increase activation of muscle precursor cells.

3. Use of NO, an NO donor, or a regulator of NO production, to increase muscle regeneration and/or repair.

4. The use according to claim 2 or 3, wherein the increase is localized to a limited area.

5. The use according to claim 2 or 3, wherein the increase is systemic.

6. Use of an NO inhibitor or a regulator of NO production to decrease activation of muscle precursor cells.

7. Use of an NO inhibitor or a regulator of NO production to decrease proliferation of muscle precursor cells.

8. The use according to claim 6 or 7, wherein the decrease is localized to a limited area.

9. The use according to claim 6 or 7, wherein the decrease is systemic.

10. A method of amplifying muscle cells in culture, comprising placing NO, an NO donor, or a regulator of NO production into contact with muscle cells.

11. A method for obtaining a muscle cell population in culture, comprising use of NO, an NO donor, or a regulator of NO production.

12. A composition comprising muscle cells and a compound selected from the group consisting of NO, an NO donor and a regulator of NO production.

13. Use of NO, an NO donor, an NO inhibitor or a regulator of NO production to modulate the effects of steroid hormone on muscle.

14. A composition comprising any one of the group consisting of NO, an NO donor, an NO inhibitor and a regulator of NO production, and a diluent or carrier suitable for use in muscle, for modulating activation of muscle precursor cells.

15. A composition comprising a compound selected from the group consisting of NO, an NO donor, an NO inhibitor and a regulator of NO production, and a component suitable for increasing concentration of the compound in muscle, for modulating activation of muscle precursor cells.

16. The composition according to claim 15 wherein the component is a muscle-targeting component.

17. The composition according to claim 16 wherein the muscle-targeting component is an antibody or an antibody fragment with binding specificity against a protein selected from the group consisting of Bcl-2, HGF, M-cadherin, HGF-activating enzyme and collagen IV.

18. A commercial package containing as an active ingredient NO, an NO donor, an NO inhibitor or a regulator of

NO production, together with instructions for its use for modulating activation of muscle precursor cells.

19. A commercial package containing as an active ingredient the composition of claim 14 or claim 15, together with instructions for its use for modulating activation of muscle precursor cells.

20. The use, method, composition or package according to any one of claims 1 to 5, 10 to 19, wherein the NO donor is selected from the group consisting of organic nitrates, organic nitrites, inorganic nitroso compounds, sydnonimines, furoxans and S-nitrosothiols.

21. The use, method, composition or package according to claim 20 wherein the NO donor is L-arginine.

22. The use, method, composition or package according to any one of claims 1, 6 to 9, 13 to 21 wherein the NO inhibitor is selected from the group consisting of N-methosyl-D-glucamine dithiocarbamate/ferrous sulfate mixture, carboxy PTIO (2-(4-carboxyphenyl) 4,4,5,5-tetra methylimidazoline-1-oxyl 3-oxide), calcium chelator BAPTA/AM, S-nitroso-N-acetylpenicillamine, 3-morpholini sydnonimine, diethyldithiocarbamate, melatonin and its precursors, superoxide dismutase, glutathione peroxidase, glutathione reductase, and dimethyl sufoxide.

23. The use, method, composition or package according to any one of claims 1 to 21 wherein the regulator of NO production is selected from the group consisting of nitric acid synthase (NOS), enhancer of NOS activity, inhibitor of NOS activity, enhancer of NOS gene expression and variants of NOS.

24. The use, method, composition or package according to any one of claims 1 to 21 wherein the regulator of NO production is nitric acid synthase NOS 1μ.

25. The use, method, composition or package according to claim 23 wherein the inhibitor of NOS activity is Nω-nitro-L-arginine methyl ester (L-NAME).

26. A method for validating a test wherein a change in activation state of muscle precursor cells is determined, comprising use of a DNA intercalator to determine that fibers associated with the precursor cells are intact.

27. A method for validating a test wherein a fiber hypercontraction-dependent change in activation state of muscle precursor cells is determined, comprising use of a myotoxin and a DNA intercalator to determine fiber membrane damage.

28. The method according to claim 26 or 27 wherein the test is a diagnostic test.

29. A method for identifying a compound which effects a change in activation state of muscle precursor cells, comprising:

(a) determining that fibers associated with the precursor cells are intact;

(b) determining the activation state of precursor cells in the absence of the compound; and

(c) determining the activation state of precursor cells treated with the compound;

wherein the difference between the two activation states identify the compound as a compound which effects a change in activation state of muscle precursor cells.

30. A method for identifying a compound which effects a fiber hypercontraction-dependent change in activation state of muscle precursor cells, comprising:

(a) treating an intact fiber containing precursor cells with a myotoxin and a DNA intercalator to effect fiber hypercontraction;

(b) determining the activation state of precursor cells in the absence of the myotoxin, DNA intercalator and the compound; and

(c) determining the activation state of precursor cells treated with the compound in the absence of the myotoxin and DNA intercalator;

wherein the difference between the two activation states identify the compound as a compound which effects a fiber hypercontraction-dependent change in activation state of muscle precursor cells.

31. The method according to any one of claims 26 to 28 and 30 wherein the DNA intercalator is ethidium bromide or propidium iodide.

32. The method according to any one of claims 27, 28, 30 and 31 wherein the myotoxin is marcaine.

33. The method according to claim 13 or 24 wherein the steroid hormone is an anabolic steroid or a glucocorticoid.

34. The method according to claim 24 wherein the steroid hormone is selected from the group consisting of deflazacort, a derivative of prednisone or a derivative of methyl-prednisone.

35. The method according to claim 24 wherein the steroid hormone is deflazacort.

36. A method for modulating muscle growth or muscle fiber branching in a vertebrate animal, comprising use of NO, an NO donor, an NO inhibitor, a regulator of NO production, or the composition according to any one of claims 12 to 15.

37. A method for regenerating muscle tissue in human dystrophy, comprising use of NO, an NO donor, an NO inhibitor, a regulator of NO production, or the composition according to any one of claims 12 to 15.

38. Method for augmenting steroid hormone treatment of human dystrophy, comprising use of NO, an NO donor, an NO inhibitor, a regulator of NO production, or the composition according to any one of claims 12 to 15.

39. The method according to claim 23 or 24 wherein the dystrophy is selected from the group consisting of Duchenne, Becker, Emery-Dreifuss, Landouzy-Dejerine, Scapulohumeral of Seitz, Limb-girdle (Erb), von Graefe-Fuchs, Oculopharyngeal, Myotonic (Steinert) and Congenital dystrophy.

40. The use, method, composition or package according to any one of claims 1 to 39, wherein the muscle is skeletal muscle.

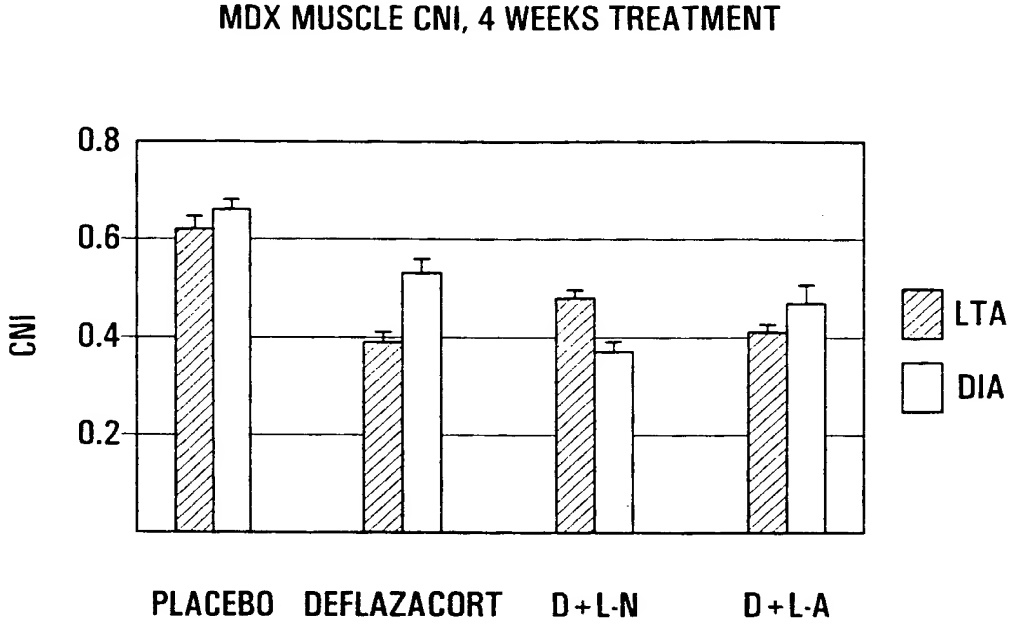

MDX MUSCLE CNI, 4 WEEKS TREATMENT
CNI
0.8
0.6
0.4
0.2
LTA
DIA
PLACEBO
DEFLAZACORT
D+L-N
D+L-A

FIG. 14

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

NGUYEN, Thuy H. et al.
SMART & BIGGAR
900-55 Metcalfe Street
P.O. Box 2999, Station D
Ottawa, Ontario K1P 5Y6
CANADA

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	29.06.2001
Applicant's or agent's file reference	IMPORTANT NOTIFICATION
74618-16	
International application No.	International filing date *(day/month/year)*
PCT/CA00/00255	10/03/2000
Priority date *(day/month/year)*	11/03/1999
Applicant	
THE UNIVERSITY OF MANITOBA et al.	

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Almalé Murillo, J-A Tel.+49 89 2399-8059

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
74618-16		
International application No. PCT/CA00/00255	International filing date *(day/month/year)* 10/03/2000	Priority date *(day/month/year)* 11/03/1999
International Patent Classification (IPC) or national classification and IPC A61K33/00		

Applicant
THE UNIVERSITY OF MANITOBA et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 32 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report
   II ☐ Priority
   III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   IV ☒ Lack of unity of invention
   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   VI ☐ Certain documents cited
   VII ☐ Certain defects in the international application
   VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/10/2000	29.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Giacobbe, S Telephone No. +49 89 2399 8463

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00255

I. Basis of the report

1. With regard to the elements of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, pages:

2,4-31,33-104	as originally filed			
105,106	with telefax of	06/10/2000		
1a,3,3a,32,32a	as received on	22/02/2001	with letter of	20/02/2001
1	with telefax of	15/06/2001		

Claims, No.:

1-76	with telefax of	15/06/2001

Drawings, sheets:

1/24-23/24	as originally filed	
24/24	with telefax of	06/10/2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT International application No. PCT/CA00/00255

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 10, 20-24, 30-59, 64, 65, 68-74.

because:

☒ the said international application, or the said claims Nos. 1-22, 30, 31, 34, 35, 38, 39, 42, 43, 46, 47, 50, 51, 54-70, 73, 74 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 10, 20-24, 30-59, 64, 65, 68-74.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT International application No. PCT/CA00/00255

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-9, 11-19, 60-63, 66 and 67.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-9, 11-19, 24-29, 60-63, 66 and 67
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-9, 11-19, 24-29, 60-63, 66 and 67
Industrial applicability (IA)	Yes:	Claims	
	No:	Claims	1-9, 11-19, 60-63, 66 and 67

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00255

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/CA00/00255

1. Section I

1.1 The amendments introduced on pages 105 and 106 and in Figure 14 do not fulfill the requirements set out in the PCT Guidelines (cf. VI-7.14) and cannot therefore be considered as correcting errors. Since moreover they completely reverse the conclusions to be drawn from the experiments, they are considered as introducing new subject-matter (see PCT Guidelines, VI-7.9) and are therefore not accepted.

1.2 The amended claims filed on 15.06.2001 do not fulfill the requirements of Art 34(2)(b) PCT since the concept of sustained skeletal muscle formation and/or repair was not present in the application as filed. The present Report has therefore been established based on the claims filed on 20.02.2001.

2. Section III

2.1 The present Opinion is based on a Partial Search Report where only claims 1-9, 14-19, 36, 37, 39 and 40 have been searched. These claims correspond to claims 1-9, 11-19, 60-63, 66 and 67 of 20.02.2001, and only these are therefore object of this Opinion (see Rules 66.1 (e) and 70.1 (d) PCT).

2.2 Claims 1-22, 30, 31, 34, 35, 38, 39, 42, 43, 46, 47, 50, 51, 54-70, 73 and 74 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT). However, although not required under the provisions of the PCT, an opinion will be given with respect to novelty and inventive step.

1. Section IV

This IPEA agrees with the objection as to lack of unity put forward by the ISA, for the reasons already given in Form PCT/ISA/206. Since the Applicant, upon invitation, has not paid any additional fee, the present Opinion will be drawn only with respect of the invention first mentioned in the application, i.e. the invention for which a Search Report has been established. This invention, concerned with the use of NO in the *in vivo* modulation of the activation of muscle precursor cells in relation to the treatment of dystrphies, is contained in claims 1-9, 11-19, 60-63, 66 and 67.

3. Section V

3.1 Cited Documents

The following documents (D) are referred to in this Report:

D1: WO 97 33173 A (UNIV CALIFORNIA) 12 September 1997

D2: US-A-5 583 101 (STAMLER JONATHAN ET AL) 10 December 1996

D3: DATABASE WPI Section Ch, Week 199831 Derwent Publications Ltd., London, GB; Class B03, AN 1998-350696, XP002154301

D4: LEE KUN HO ET AL, JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 20, 1994, pages 14371-14374, XP002154298

D5: DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US1993 BAEK MI-YEONG ET AL, XP002154299

D6: BREDT DAVID S, PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 25, 1998, pages 14592-14593, XP000960480

3.2 Art 33(2) PCT (Novelty)

The subject-matter of present claims 1-9, 11-19, 24-29, 60-63, 66 and 67 does not meet the requirements of Art 33(2) PCT.

3.2a Document D1 discloses that NO and neuronal NO synthase can be used for the treatment of muscular dystrophies (cf. abstract; p. 2, ll. 1-12; and p. 11, ll. 15-26). This document is therefore novelty-destroying for claims 1-9, 11-19, 60-63, 66 and 67

n.b. The fact that NO and NO synthase are disclosed in D1 to act by a mechanism which has nothing to do with the activation of satellite cells is irrelevant, since not the mechanism of action but rather the treated diseases define the invention. In other words, the discovery of a new mechanism of action of a known substance used in the state of the art to treat a given disease is not a patentable invention unless it solves a specific technical problem (e.g. a specific time course of the treatment) over the same prior art. In such a case however the technical feature allowing the solution of this technical problem must be present in the application as filed and indicated in the claims; the mechanism of action does not constitute such a technical feature.

3.2b Document D2 discloses that pharmaceutical compositions containing NO synthase

inhibitors (cf. cols. 2-6) can be used for the treatment of muscular dystrophies (cf. col. 8, l. 15). This document is therefore (cf. n.b. above) novelty-destroying for claims 1-9, 11-19, 24-29, 60-63, 66 and 67.

3.2c Document D3 discloses that pharmaceutical compositions containing NO synthase inhibitors can be used for the treatment of muscular dystrophies. This document is therefore (cf. n.b. above) novelty-destroying for claims 1-9, 11-19, 24-29, 60-63, 66 and 67.

3.3 Art 33(3) PCT (Inventive step)

The subject-matter of present claims 1-9, 11-19, 24-29, 60-63, 66 and 67 does not meet the requirements of Art 33(2) PCT.

Document D4 (cf. p. 14371, col. 2, first complete paragraph) explicitly states that "the fusion of mononucleated myoblasts (i.e. of myogenic precursor cells or satellite cells as these cells are called in the present description, p. 1) into multinucleated myotubules (i.e. of myofibers, cf again the present description, p. 1)" constitutes a prominent event in the differentiation of embryonic muscle cells, an event which is shown in D4 itself, as well as in D5, to be mediated by NO. Furthermore it was known at the date of first filing of the present application that "muscle repair and formation are enabled by satellite (i.e. myoblast) activation" (cf. present description, p. 5, ll. 5-7). The skilled person could have therefore come to the logical conclusion that NO, by mediating myoblast fusion, could solve the technical problem of how to promote muscle repair and formation. This is confirmed by the conclusion contained in D6 that "manipulating NO levels in muscle may represent a possible strategy for the treatment of muscular dystrophy" (cf. p. 14593, last sentence).

3.4 Art 33(4) PCT (Industrial applicability)

As stated above, no opinion is given on the question of whether present claims 1-9, 11-19, 60-63, 66 and 67 are industrially applicable since their patentability is inter alia dependent upon their formulation as well as upon national and regional laws and no unifying criteria is provided in this field by the PCT.

4. Section VIII

Independent claims 1-3, 11-13 and 60-63 are not clear because they define the subject-matter to be protected by way of the biological mechanism underlying the

action of the disclosed compounds. If a claim is directed to a condition susceptible of being improved or prevented by selective interaction with a biological pathway, the claim can be regarded as clear only if instruction, in the form of experimental tests or any testable criteria, allowing the skilled person to recognise which conditions fall within the functional definition (and accordingly within the scope of the claims concerned) are available from the patent documents or from the general common knowledge. The selective interaction with a biological pathway itself cannot be considered as a therapeutic application. In the absence of such tests or criteria, a clear indication of the diseases to be treated is required in order to fulfill the requirements of Art 6 PCT. The claims have been examined under the assumption that the diseases indicated in claims 66 and 67 are intended.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 33/00

A2

(11) International Publication Number: WO 00/53191

(43) International Publication Date: 14 September 2000 (14.09.00)

(21) International Application Number: PCT/CA00/00255

(22) International Filing Date: 10 March 2000 (10.03.00)

(30) Priority Data: 60/123,895 11 March 1999 (11.03.99) US

(71) Applicant (*for all designated States except US*): THE UNIVERSITY OF MANITOBA [CA/CA]; Room 202, Administration Building, 66 Chancellors Circle, Winnipeg, Manitoba R3T 2N2 (CA).

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): ANDERSON, Judy, E. [CA/CA]; 189 Kingsway Avenue, Winnipeg, Manitoba R3M 0G4 (CA).

(74) Agents: WHEELER, Michael, E. et al.; Smart & Biggar, 900–55 Metcalfe Street, P.O. Box 2999, Station D, Ottawa, Ontario K1P 5Y6 (CA).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

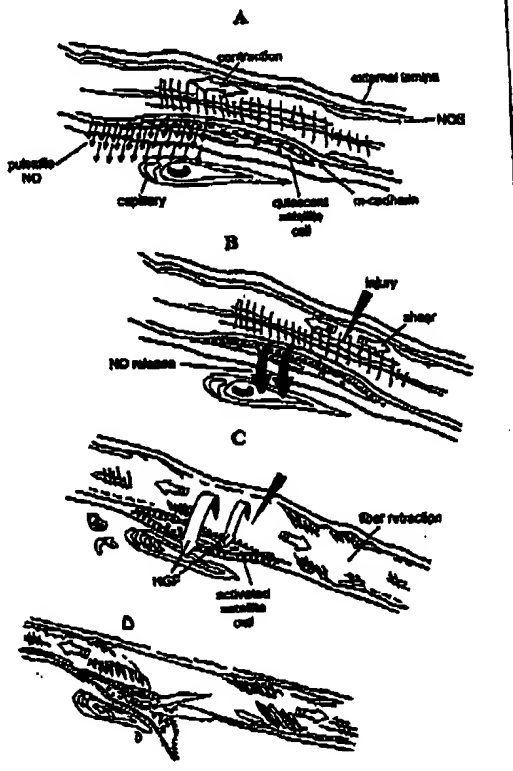
Published

Without international search report and to be republished upon receipt of that report.

(54) Title: MODULATION OF SKELETAL MUSCLE PRECURSOR CELL ACTIVATION

(57) Abstract

The present invention is directed to methods, pharmaceutical compositions and kits for modulating skeletal muscle precursor cell activation. Modulation is effected through the use of nitric oxide (NO), donors of NO, inhibitors of NO activity (NO inhibitor) or regulators of NO production, either locally or systemically. The invention further teaches the use of NO, an NO donor, an NO inhibitor or a regulator of NO production to modulate the effects of steroid hormone on skeletal muscle. The invention further provides a method for identifying a compound which effects a change in activation state of muscle precursor cells. A number of advantages is evident. By allowing skeletal muscle precursor cells to be manipulated directly, the invention enables specific treatments to regenerate and repair muscle.



INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/CA 00/00255

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/04 A61K31/195 A61K31/295 A61K31/70 A61K31/415
A61K31/535 A61K31/145 A61K31/40 A61K31/10 A61K38/44
A61K35/34 C12N5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ULIBARRI J A ET AL: "Nitric oxide stimulates myoblast proliferation in vitro." MEDICINE AND SCIENCE IN SPORTS AND EXERCISE, vol. 29, no. 5 SUPPL., 1997, page S228 XP000961780 44th Annual Meeting of the American College of Sports Medicine;Denver, Colorado, USA; May 28-31, 1997 ISSN: 0195-9131 abstract --- -/--	1-12,36, 40

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 December 2000	19.03.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	A. Jakobs

Form PCT/ISA/210 (second sheet) (July 1992)

ISA/EP

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US1993 BAEK MI-YEONG ET AL: "Changes in the cellular cGMp levels and guanylate cyclase activities during chick myoblast fusion." Database accession no. PREV199396097003 XP002154299 abstract & KOREAN JOURNAL OF ZOOLOGY, vol. 36, no. 3, 1993, pages 433-438, ISSN: 0440-2510 ---	1-9, 14-19, 36,37,40
X	BREDT DAVID S: "NO skeletal muscle derived relaxing factor in Duchenne muscular dystrophy." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 95, no. 25, December 1998 (1998-12), pages 14592-14593, XP000960480 Dec., 1998 ISSN: 0027-8424 page 14592, column 2, paragraph 3 -page 14593, column 1, paragraph 5 ---	1
X	SARKAR RAJABRATA ET AL: "Nitric oxide inhibition of endothelial cell mitogenesis and proliferation." SURGERY (ST LOUIS), vol. 118, no. 2, 1995, pages 274-279, XP000961764 ISSN: 0039-6060 abstract ---	1
X	DATABASE WPI Section Ch, Week 199831 Derwent Publications Ltd., London, GB; Class B03, AN 1998-350696 XP002154301 & JP 10 120654 A (ONO PHARM CO LTD), 12 May 1998 (1998-05-12) abstract --- -/--	1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US November 1997 (1997-11) LAMOSOVA D ET AL: "Influence of melatonin on chick skeletal muscle cell growth." Database accession no. PREV199800098087 XP002154300 abstract & COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY C PHARMACOLOGY TOXICOLOGY &, vol. 118, no. 3, November 1997 (1997-11), pages 375-379, Nov., 1997 ISSN: 0742-8413 ---	1
X	AZZENA G B ET AL: "NITRIC OXIDE REGENERATES THE NORMAL COLONIC PERISTALTIC ACTIVITY IN MDX DYSTROPHIC MOUSE" NEUROSCIENCE LETTERS,LIMERICK,IE, vol. 261, no. 1/02, 1999, pages 9-12, XP000879028 ISSN: 0304-3940 the whole document ---	1-9, 14-19, 36,37, 39,40
X	LEE KUN HO ET AL: "Nitric oxide as a messenger molecular for myoblast fusion." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 20, 1994, pages 14371-14374, XP002154298 ISSN: 0021-9258 abstract; figures 1,4 page 14372, column 2, paragraph 3 ---	1-9, 14-19, 36,37,40
X	YAN ZHONG-QUN ET AL: "Overexpression of inducible nitric oxide synthase by neointimal smooth muscle cells." CIRCULATION RESEARCH, vol. 82, no. 1, pages 21-29, XP000961767 ISSN: 0009-7330 abstract page 24, column 2, paragraph 5 -page 26, column 2, paragraph 1; figures 7,8 page 28, column 2, paragraphs 2,3 --- -/--	1-9, 14-19, 36,37,40

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HAYCOCK J W ET AL: "OXIDATIVE DAMAGE TO MUSCLE PROTEIN IN DUCHENNE MUSCULAR DYSTROPHY" NEUROREPORT,GB,RAPID COMMUNICATIONS OF OXFORD, OXFORD, vol. 8, no. 1, 1996, pages 357-361, XP000879014 ISSN: 0959-4965 abstract page 357, column 2, paragraph 2 -page 358, column 1, paragraph 1 page 361, column 1, paragraph 2 -column 2, paragraph 2 ---	1-9, 14-19, 36,37, 39,40
X	CHAO DANIEL S ET AL: "Selective loss of sarcolemmal nitric oxide synthase in becker muscular dystrophy." JOURNAL OF EXPERIMENTAL MEDICINE, vol. 184, no. 2, 1996, pages 609-618, XP000961763 ISSN: 0022-1007 abstract page 610, column 1, paragraphs 2,3 table 1 page 616, column 2, paragraphs 2,3 ---	1-9, 14-19, 36,37, 39,40
X	AZZENA GIAN BATTISTA ET AL: "Nitric oxide regenerates the normal colonic peristaltic activity in mdx dystrophic mouse." NEUROSCIENCE LETTERS, vol. 261, no. 1-2, 12 February 1999 (1999-02-12), pages 9-12, XP000961771 ISSN: 0304-3940 abstract page 9, column 1 -page 10, column 1, paragraph 1 page 12, column 1 ---	1-9, 14-19, 36,37, 39,40
X	US 5 583 101 A (STAMLER JONATHAN ET AL) 10 December 1996 (1996-12-10) abstract; examples 1-6 column 1 -column 6, line 40 column 8, paragraph 2 ---	1-9, 14-19, 36,37, 39,40

-/--

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/CA 00/00255

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SOHN YOON K ET AL: "Neuritic sprouting with aberrant expression of the nitric oxide synthase III gene in neurodegenerative diseases." JOURNAL OF THE NEUROLOGICAL SCIENCES, vol. 162, no. 2, 15 January 1999 (1999-01-15), pages 133-151, XP000961766 ISSN: 0022-510X the whole document ---	1-9, 14-19, 36,37, 39,40
A	WO 97 33173 A (UNIV CALIFORNIA) 12 September 1997 (1997-09-12) the whole document ---	1-40
P,X	KALIMAN, PERLA ET AL: "Insulin-like growth factor-II, phosphatidylinositol 3-kinase, nuclear factor-.kappa.B and inducible nitric-oxide synthase define a common myogenic signaling pathway" J. BIOL. CHEM. (1999), 274(25), 17437-17444, XP000960874 the whole document ---	1-9, 14-19, 36,37,40
A	EL-DADA, MANAR D. ET AL: "Involvement of nitric oxide in nicotinic receptor-mediated myopathy" J. PHARMACOL. EXP. THER. (1997), 281(3), 1463-1470, XP000972194 the whole document -----	1-40

INTERNATIONAL SEARCH REPORT

Int...ational application No.
PCT/CA 00/00255

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9, 14-19, 36, 37, 39, 40 (all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interna[illegible]al Application No
PCT/CA 00/00255

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 10120654 A	12-05-1998	NONE	
US 5583101 A	10-12-1996	AU 3008395 A	16-02-1996
		CA 2194991 A	01-02-1996
		JP 10511075 T	27-10-1998
		WO 9602241 A	01-02-1996
		US 5545614 A	13-08-1996
WO 9733173 A	12-09-1997	AU 2208097 A	22-09-1997

INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/CA 00/00255

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/04 A61K31/195 A61K31/295 A61K31/70 A61K31/415
A61K31/535 A61K31/145 A61K31/40 A61K31/10 A61K38/44
A61K35/34 C12N5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ULIBARRI J A ET AL: "Nitric oxide stimulates myoblast proliferation in vitro." MEDICINE AND SCIENCE IN SPORTS AND EXERCISE, vol. 29, no. 5 SUPPL., 1997, page S228 XP000961780 44th Annual Meeting of the American College of Sports Medicine;Denver, Colorado, USA; May 28-31, 1997 ISSN: 0195-9131 abstract --- -/--	1-12,36, 40

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 December 2000	19.03.01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	A. Jakobs

Form PCT/ISA/210 (second sheet) (July 1992)

ISA/EP

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

	Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
1	X	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US November 1997 (1997-11) LAMOSOVA D ET AL: "Influence of melatonin on chick skeletal muscle cell growth." Database accession no. PREV199800098087 XP002154300 abstract & COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY C PHARMACOLOGY TOXICOLOGY &, vol. 118, no. 3, November 1997 (1997-11), pages 375-379, Nov., 1997 ISSN: 0742-8413 ---	1
4	X	AZZENA G B ET AL: "NITRIC OXIDE REGENERATES THE NORMAL COLONIC PERISTALTIC ACTIVITY IN MDX DYSTROPHIC MOUSE" NEUROSCIENCE LETTERS,LIMERICK,IE, vol. 261, no. 1/02, 1999, pages 9-12, XP000879028 ISSN: 0304-3940 the whole document ---	1-9, 14-19, 36,37, 39,40
1	X	LEE KUN HO ET AL: "Nitric oxide as a messenger molecular for myoblast fusion." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 20, 1994, pages 14371-14374, XP002154298 ISSN: 0021-9258 abstract; figures 1,4 page 14372, column 2, paragraph 3 ---	1-9, 14-19, 36,37,40
1	X	YAN ZHONG-QUN ET AL: "Overexpression of inducible nitric oxide synthase by neointimal smooth muscle cells." CIRCULATION RESEARCH, vol. 82, no. 1, pages 21-29, XP000961767 ISSN: 0009-7330 abstract page 24, column 2, paragraph 5 -page 26, column 2, paragraph 1; figures 7,8 page 28, column 2, paragraphs 2,3 --- -/--	1-9, 14-19, 36,37,40
3			

INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/CA 00/00255

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	SOHN YOON K ET AL: "Neuritic sprouting with aberrant expression of the nitric oxide synthase III gene in neurodegenerative diseases." JOURNAL OF THE NEUROLOGICAL SCIENCES, vol. 162, no. 2, 15 January 1999 (1999-01-15), pages 133-151, XP000961766 ISSN: 0022-510X the whole document	1-9, 14-19, 36,37, 39,40
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A	EL-DADA, MANAR D. ET AL: "Involvement of nitric oxide in nicotinic receptor-mediated myopathy" J. PHARMACOL. EXP. THER. (1997), 281(3), 1463-1470, XP000972194 the whole document	1-40

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat Application No
PCT/CA 00/00255

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 10120654 A	12-05-1998	NONE	
US 5583101 A	10-12-1996	AU 3008395 A	16-02-1996
		CA 2194991 A	01-02-1996
		JP 10511075 T	27-10-1998
		WO 9602241 A	01-02-1996
		US 5545614 A	13-08-1996
WO 9733173 A	12-09-1997	AU 2208097 A	22-09-1997

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 06 November 2000 (06.11.00)	
International application No. PCT/CA00/00255	**Applicant's or agent's file reference** 74618-16
International filing date (day/month/year) 10 March 2000 (10.03.00)	**Priority date** (day/month/year) 11 March 1999 (11.03.99)
Applicant ANDERSON, Judy, E.	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

06 October 2000 (06.10.00)

[ ] in a notice effecting later election filed with the International Bureau on:

_____

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Charlotte ENGER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38